Clinical Investigation

Clinical Responses With Active Specific Intralymphatic Immunotherapy for Cancer—A Phase I-II Trial

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We evaluated the method of active specific intralymphatic immunization to treat cancer in 32 patients with various tumor types as part of a broad-based phase I-II evaluation and describe the results of 3 sequential series. In series 1, the patients (n = 13) received 2 or more injections of autologous, cryopreserved, irradiated tumor cells directly into the lymphatic system through the cannulation of a dorsal pedal lymphatic channel. In series 2, the patients (n = 7) received low-dose cyclophosphamide, 300 mg per m², 3 days before the autologous cell vaccine was administered. Series 3 (12 patients) was similar to series 2 except that the tumor cells were treated with cholesteryl hemisuccinate immediately before irradiation. Patients received from 2 to 6 injections of cells, depending on availability, at 2-week intervals. In all, 91 treatments are evaluated in this study. Clinical responses occurred in 7 of the 32 patients and were seen in all 3 series with about the same frequency. These responses occurred in cases of melanoma, lung cancer, colon cancer, and sarcoma. Regressions occurred in both visceral and subcutaneous sites. There was little toxicity, the chief side effect being local discomfort or inflammation. This experience indicates that active specific intralymphatic immunotherapy is safe, produces antitumor effects, and requires more investigation to increase the frequency and duration of observable tumor regression.

(Wiseman CL, Rao VS, Kennedy PS, et al: Clinical responses with active specific intralymphatic immunotherapy for cancer—A phase I-II trial. West J Med 1989 Sep; 151:283-288)

The historical enthusiasm for using autologous tumor cells as immunogens has resulted in a number of clinical trials, and some positive results warrant further attention.[1] We have been interested in several recent developments that encourage further investigation of such an approach. We began a study to investigate and extend the original report of Juillard and co-workers, who described regression of metastatic tumors after inoculating irradiated tumor cells directly into the lymph nodes through the dorsal pedal lymphatic vessels.[2]

Direct intralymphatic immunization is an attractive concept for a number of theoretic reasons. Such a technique could circumvent possible immunosuppressive mechanisms of a primary tumor. Regional lymph nodes vary widely in immunologic responsiveness.[3] Some studies indicate that nodes close to a primary tumor have decreased functional competence and increased suppressor-cell activity as compared with nodes distant from the primary tumor.[4]

The intralymphatic approach has been studied in animals[5,6] with positive results and in several human tumor categories. In addition to the work of Juillard and colleagues, augmented immunologic responses were reported in breast cancer and in renal cancer by Adler and associates, although the clinical significance of these responses remains open.[7,8]

Our initial findings suggested that the method reproducibly elicited substantial biologic effects, with a significant elevation of the fraction (and absolute number) of circulating CD4+ lymphocytes. We observed that this elevation occurred after each immunization and in almost every patient.[9,10] In a few melanoma patients, two subpopulations of CD4+ were identified.[11]

Since the initiation of this study, we have made several modifications based on possibly useful newer techniques. The reports by Berd and Mastrangelo indicated that the use of low doses of cyclophosphamide may selectively block suppressor-cell functional activity, thereby leading to augmented specific immune responses.[12] After our initial experience, we introduced this as a pretreatment for our patients (series 2 and 3). We then changed the program in an attempt to increase potential cell-surface immunogenicity of tumor cells by reducing membrane-lipid microviscosity. We introduced the methods of Skornick and associates, using a brief incubation with cholesteryl hemisuccinate (series 3).[13]

Patients and Methods

Tumor Vaccine

Surgically removed tumor was dispersed by collagenase and deoxyribonuclease according to methods previously de-

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This work was supported by the St Vincent Medical Center and the Wilshire Oncology Medical Group.

Funding in part was provided by the Auxiliary of the St Vincent Medical Center, the Ahmanson Foundation, the Zwick Fund, and the Los Angeles Oncologic Institute, a nonprofit philanthropic and research agency.

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scribed.[9] The cells were frozen slowly using a programmed cryogenic freezer (Union Carbide, Indianapolis) and stored over liquid nitrogen. On demand, the cells were thawed rapidly and washed in Hanks' medium. Adherent cells were removed by incubating at 37°C, and the remaining population was then irradiated to 200 gray from a cobalt 57 source. Dosimetry and supervision of the radiation procedure were provided by Armand Bouzaglou, MD, and John Sevilla, MD. For patients in series 3, vaccine preparation was modified. After thawing and washing, the cells were suspended in a polyvinyl pyrolidone-Hanks' solution, pH 7.2, containing 250 μg per ml cholesteryl hemisuccinate (Sigma), adjusted to 1×10^6 viable cells per milliliter, and incubated three to four hours at room temperature, washed, and resuspended in lactated Ringer's solution 5 to 10×10^6 viable cells per milliliter before irradiation. The irradiated cells were suspended in a lactated Ringer's solution at a concentration of 10 to 15 $\times 10^6$ viable cells per milliliter. Viability was assessed by trypan blue exclusion. Aliquots of the initial preparation and the irradiated vaccine were routinely tested for bacterial contamination by culture as recommended by federal protocol.[14] The viability of the tumor cells was generally about 70% or higher, although occasionally a patient was treated with a vaccine of lower viability.

Patients

All patients provided signed informed consent before enrolling in this program. Approval and periodic review had been provided by the Institutional Review Board of the St Vincent Medical Center (Los Angeles), both initially and after the sequential modifications of the program. All patients received a complete medical history and physical examination, complete blood counts, biochemical profile, and such x-ray films, computed tomograms, and isotope scans as needed to evaluate the extent of metastatic involvement and the dimensions of at least one measurable indicator lesion. Therapy was not initiated until at least three weeks had elapsed from previous chemotherapy, radiation therapy, or surgical procedure requiring general anesthesia. No additional antitumor therapy was permitted for any patient during the period of treatment on this protocol.

Autologous irradiated tumor cells, suspended at a concentration of 10 to 15 $\times 10^6$ viable cells per milliliter, were injected over several minutes into a dorsal pedal lymphatic

TABLE 1.—*Clinical Characteristics of 32 Patients Receiving Active Specific Intralymphatic Immunotherapy*

Patient	Age, yr	Sex	Primary Cancer	ECOG Scale of Metastases*	Site(s) of Metastases	Previous Chemotherapy Regimens, No.
Series 1						
1	58	♂	Melanoma	1	Lung	0
2	45	♂	Melanoma	1	Lung, liver	0
3	76	♂	Melanoma	1	Nodes	3
4	26	♂	Melanoma	4	Skin	3
5	74	♂	Melanoma	4	Lung, liver	0
6	44	♂	Colon	4	Nodes, ascites, liver	1
7	26	♂	Colon	2	Liver	0
8	75	♀	Colon	2	Liver	1
9	54	♂	Lung	2	Lung	0
10	35	♂	Lung	1	Lung	0
11	41	♂	Nasopharynx	4	Liver, bone, lung	3
12	64	♂	Renal	1	Lung, chest wall	0
13	61	♂	Renal	2	Kidney	0
Series 2						
14	61	♂	Colon	2	Lung, abdomen	1
15	46	♂	Colon	1	Liver	0
16	32	♂	Melanoma	3	Liver, lung	0
17	46	♀	Melanoma	1	Skin	2
18	42	♀	Melanoma	2	Abdomen, nodes	1
19	48	♂	Renal	2	Lung	0
20	41	♂	Unknown	3	Abdomen, lung	1
Series 3						
21	64	♀	Colon	2	Liver, bone	1
22	52	♀	Lung	4	Skin, lung, kidney	1
23	78	♂	Lung	3	Lung, liver	0
24	68	♂	Melanoma	2	Abdomen	1
25	45	♂	Melanoma	2	Abdomen, nodes	3
26	56	♂	Melanoma	3	Scalp, liver, nodes	3
27	36	♀	Melanoma	2	Lung, nodes	0
28	32	♂	Melanoma	1	Lung	3
29	62	♂	Melanoma	1	Lung, skin	1
30	45	♀	Melanoma	3	Lung	2
31	65	♂	Renal	4	Bone, lung	3
32	45	♀	Sarcoma	2	Skin, liver	1

ECOG=Eastern Cooperative Oncology Group

*The ECOG scale measures the performance status of a patient using a scale of 0 (fully active, able to carry on all predisease activities without restrictions) to 4 (completely disabled, totally confined to bed or chair).

channel isolated after Evans blue was infiltrated and lidocaine hydrochloride was administered for local anesthesia according to methods used for lymphangiography. All cutdown procedures were done by the staff of the Department of Radiology, St Vincent Medical Center. Patients received the vaccine at two- to four-week intervals as supplies permitted. Unless required for other medical considerations, admission to hospital was not needed. For those patients receiving cyclophosphamide (Cytoxan, Bristol-Myers Oncology Division), the drug was injected intravenously three days before vaccine inoculation at a dose of 300 mg per m^2 following pretreatment with the intramuscular administration of triethylperazine, 10 to 15 mg (Torecan, Roxane Laboratories, Inc).

TABLE 2.—*Distribution of Tumor Types Per Series*

Tumor Types	Patients, No.			
	Series 1	Series 2	Series 3	Total
Melanoma	5	3	7	15
Colon cancer	3	2	1	6
Lung cancer	2	0	2	4
Renal cancer	2	1	1	4
Miscellaneous*	1	1	1	3
Total	13	7	12	32

*Nasopharyngeal 1, adenocarcinoma of unknown primary 1, atrial myxosarcoma 1.

Results

The roster of patients, together with clinical characteristics, is provided in Table 1. As mentioned, our initial experience, series 1, represents the tumor vaccine program unmodified. Series 2 and 3 differ in that patients were treated with low-dose cyclophosphamide. Series 3 comprises those patients whose vaccine underwent pretreatment with cholesteryl hemisuccinate; additionally, series 3 patients, similar to the patients in series 2, received a priming, immunomodulating dose of cyclophosphamide before the vaccine treatment. There were 13 patients in series 1, 7 in series 2, and 12 in series 3. The program was initiated June 1, 1981; we evaluated responses and survival as of July 15, 1988. Table 2 shows the tumor types and their distribution according to series.

All patients had advanced cancer, usually with pulmonary or intra-abdominal metastases. The median age was 48 years (range, 26 to 78). There were 8 women. Eleven patients had had no previous therapy; an occasional patient had had extensive previous treatment. The median performance score on the Eastern Cooperative Oncology Group scale was 2; six had scores of 4 (completely bedridden) and five had

TABLE 3.—*Therapy and Responses*

Patient	Primary Cancer	Viable Cells/Cycle, × 10^6/ml	Tumor Response	Time to Progression, wk	Survival, wk	Comment
Series 1						
1	Melanoma	5.0, 25.0, 50.0, 56.0	Mixed	12.0	46.1	Partial remission, pulmonary; CNS failure
2	Melanoma	20.0, 15.0	Progression	4.0	13.0	
3	Melanoma	10.0, 14.0, 20.0	Progression	10.0	146.7	
4	Melanoma	20.0, 40.0	Progression	9.1	9.1	
5	Melanoma	10.0, 5.0, 35.0, 60.0, 20.0	Progression	12.3	13.1	
6	Colon	3.0, 6.0	Mixed	4.0	7.7	Regression of Virchow's node
7	Colon	6.0, 4.5, 4.8	Stable	19.7	24.0	
8	Colon	6.0, 9.0, 33.0	Stable	32.4	43.3	
9	Lung	10.0, 20.0, 12.0	Progression	9.0	19.1	
10	Lung	10.0, 2.0	Complete remission	22.7	43.1	See text
11	Nasopharynx	14.0, 8.0, 1.5	Progression	7.7	14.4	
12	Renal	20.0, 10.0, 30.0	Progression	10.0	299.4	
13	Renal	14.0, 14.0, 17.0	Progression	6.0	6.0	
Series 2						
14	Colon	10.0, 12.0, 10.0	Mixed	14.3	73.9	See text
15	Colon	11.0, 14.0, 14.0	Progression	8.3	169.4	Active, slowly progressive disease
16	Melanoma	20.0, 18.0	Progression	3.6	37.7	
17	Melanoma	16.0, 21.0, 12.5	Complete remission	67.9	79.0	See text
18	Melanoma	24.0, 28.0, 30.0	Stable	13.4	21.9	
19	Renal	12.0, 12.0, 13.0, 3.0, 7.0, 8.0	Progression	6.9	45.7	
20	Unknown	8.0, 9.0	Progression	7.0	9.9	
Series 3						
21	Colon	11.0, 6.0	Progression	7.0	15.6	
22	Lung	10.0, 9.7	Progression	4.6	6.1	
23	Lung	9.0, 9.0	Progression	7.0	19.3	
24	Melanoma	13.0, 9.0, 9.0	Progression	19.0	57.7	
25	Melanoma	10.0, 10.0, 10.0	Progression	10.0	59.3	
26	Melanoma	10.0, 12.0	Progression	5.7	6.4	
27	Melanoma	8.0, 8.0	Progression	12.4	17.1	
28	Melanoma	13.0, 13.0, 12.0, 10.0	Progression	9.0	38.7	
29	Melanoma	10.0, 10.0, 11.0	Partial remission	25.1	56.7	
30	Melanoma	8.0, 9.0, 9.0	Progression	6.9	13.1	
31	Renal	17.0, 16.0	Progression	4.4	11.9	
32	Sarcoma	11.0, 12.0, 14.0	Mixed	5.3	42.9	See text

CNS=central nervous system

scores of 3—that is, about a third of the patients were partially or completely bedridden.

Table 3 depicts the number of cycles of immunization per patient, the amount of viable cells of each vaccine treatment, the maximum response according to standard criteria of the Southwestern Oncology Group, the time to progression, and the duration of survival from the initiation of treatment. For 32 patients in the study, there were a total of 91 treatments. Examples of objective responses are depicted in Figures 1, 2, and 3. Figure 1 shows a chest x-ray film of patient 10, a 35-year-old man who underwent an exploratory thoracotomy for operable but not resectable, large-cell undifferentiated lung cancer. The patient received two vaccine treatments, with the subsequent disappearance of the lung nodule and improvement of mediastinal widening, both on chest x-ray film and computed tomography (CT). The patient was then treated by his referring physician with chemotherapy and irradiation to consolidate the remission, but the disease recurred five months later.

Figure 2 shows the computed tomograms of patient 29, a 62-year-old man who had an excisional biopsy of pulmonary nodules diagnostic of metastatic melanoma. In addition to pulmonary metastases, the patient had noticed a 1-cm nodule in the right thigh shortly before the initiation of vaccine treatment. The pulmonary disease showed regression, the thigh mass disappeared, and the patient had a reduction of serum concentrations to the monoclonal antibody CA 125 from 91 to 33 ng per ml eight weeks following the start of treatment. The patient then showed progressive disease in the lung and also brain metastases after six months.

Figure 3 shows CT scans taken of a 45-year-old woman (patient 32) who presented with an atrial myxoma invading the great vessels. After surgical resection she was treated with infusional chemotherapy with doxorubicin (Adriamycin, Adria Laboratories) hydrochloride, but hepatic metastases developed. She received three cycles of autologous irradiated tumor cell vaccine as per the methods described for series 3 patients. The follow-up scans five weeks later showed disappearance of several vaguely demarcated liver lesions and reduction of a major lesion from 4 by 4 cm to 1.5 by 1.5 cm. New lesions were identified on the skin and also in the lungs, but the hepatic lesions regressed.

Patient 1 had multiple vaguely defined pulmonary infiltrates, which showed more than 50% regression simultaneous with the development of brain metastases. Patient 14 had an exploratory laparotomy with partial resection of a retroperitoneal mass, not identifiable on computed tomography. Signs of a bowel obstruction developed 14 weeks later, again without tumor visible by CT scan. At an operation the patient had tumor obstructing the distal small bowel but the surgeon saw that the original tumor mass was more than 50% reduced.

Patient 17 had a resection of one of several large breast masses palpable and visible on mammography. After three vaccine treatments, the mass adjacent to the resected tumor appeared to enlarge and was resected. Interestingly, only necrotic debris was obtained. When the patient relapsed 68 weeks later, there was a large, palpable, and darkly pigmented nodule in the region from which a biopsy was previously taken, as well as several other areas in both breasts.

The median survival for all patients was 36 weeks. We note that occasionally a patient had an unusually long survival. Patient 12, with recurrent renal carcinoma in the chest wall, showed a greater than 25% increase in the size of the tumor; he was subsequently treated with a regimen of interferon, without significant benefit, then radiation therapy. Lung metastases also developed. Nonetheless, he remains ambulatory and reasonably active 299 weeks after the autologous intralymphatic vaccine therapy started. Patient 15 had progressive liver metastases on a CT scan after three treatments with autologous irradiated tumor cells and was treated with two courses of intrahepatic chemotherapy with mito-

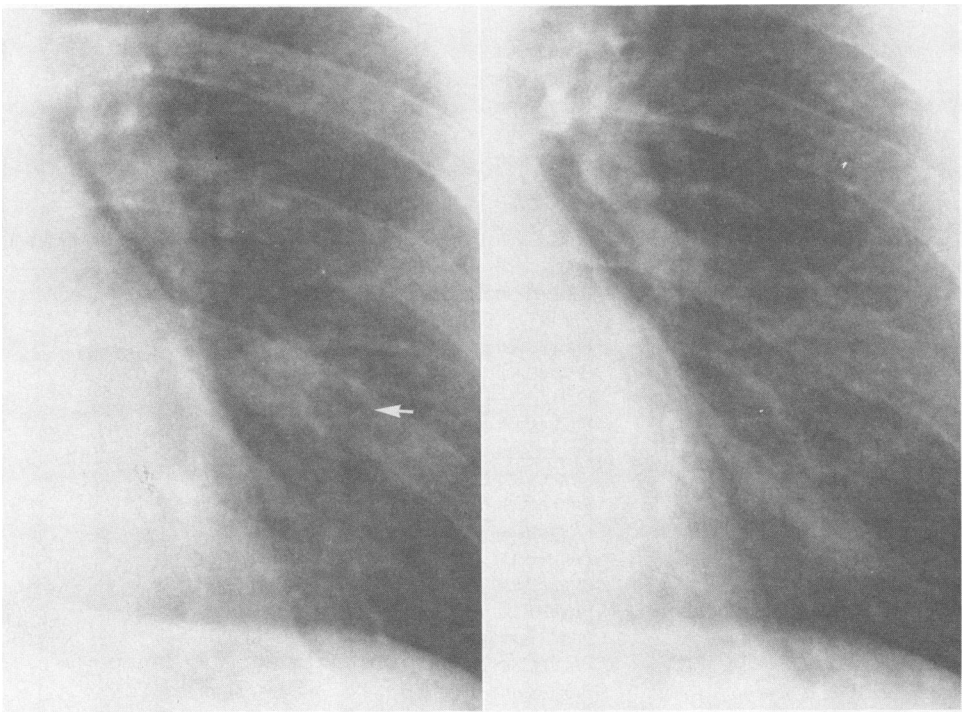

Figure 1.—Left, A chest x-ray film of patient 10 before vaccine therapy shows a lesion **(arrow)** in the left hilum. **Right,** After 2 cycles, the left hilar lesion has disappeared.

mycin and fluorouracil. He has declined further medical evaluation but continues to work more than 169 weeks since treatment was initiated.

Direct toxicity was limited to an occasional local infection, rarely fever or other systemic reactions. There were no instances of regional adenopathy, nor did any patient have symptoms of immediate hypersensitivity. There was no evidence of tumor enhancement or acceleration of the clinical course. Wound infections and difficult cannulations were seen primarily in series 1 patients (three patients each). Fever was identified briefly in one patient, who received antipyretics and had a normal temperature after 24 hours. One patient (number 8) with colon carcinoma and extensive pelvic tumor had a self-limited deep vein thrombophlebitis in the contralateral leg; this condition required admission to hospital and anticoagulation but resolved without sequelae. The patient had no further complications until her demise from progressive distant metastases 32 weeks later. Another patient (number 13) with renal carcinoma died suddenly at home; at autopsy a pulmonary embolus was identified. This event is not unexpected in this population, but there is a possibility that the cutdown procedure, requiring immobilization on a guerney for 60 to 120 minutes, contributed to its development. Nonetheless, patients in the later series were not similarly affected.

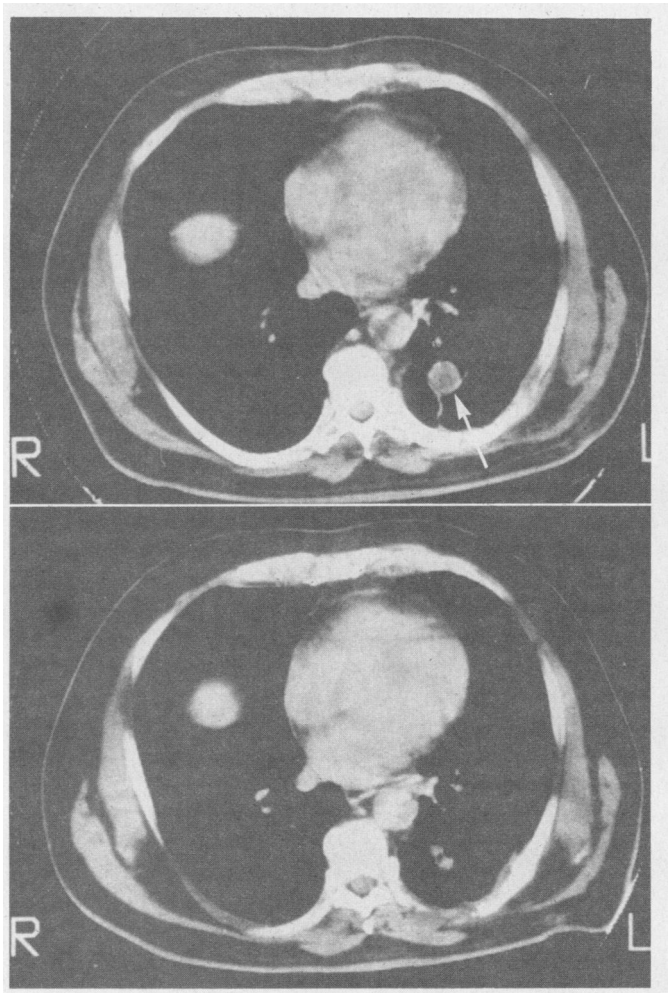

Figure 2.—Top, A computed tomogram at the start of treatment in patient 29 shows a large pulmonary nodule **(arrow)** in the left posterior lung. **Bottom,** A computed tomogram taken 60 days later shows regression of the nodule in the left posterior lung.

Discussion

This report confirms the clinical interest of active specific immunotherapy using autologous cells through intralymphatic injection. The original report by Juillard and colleagues in 1978 showed that tumor vaccines could elicit regressions of metastatic disease,[2] a controversial notion at a time when it was widely held that immunologic mechanisms would be ineffective except for subclinical, micrometastatic disease.[1] We describe here objective regressions in 7 of 32 patients studied. It should be noted that these very ill patients all had tumors for which effective or even palliative therapy is marginal. The responses have been limited and incomplete, however, and the problem of mixed responses is disappointing. McCune and co-workers, in a study of renal carcinoma, interpret mixed responses as a strong argument for the polyclonality of tumors.[15] The response of patient 32 is encouraging, even if there was growth of tumor at other sites, given the severity of liver involvement and the rarity of regressions under these circumstances.

In evaluating the population for possible prognostic factors, we note that all of the responding patients were younger than 65 and most had performance status scores of 2 or less. The mean age of the seven responders, however, was 50, and that age was not appreciably different from that of the group as a whole (mean age, 51.3 years).

Previous chemotherapy was not a contraindication to a response, contrary to the experience of Weisenburger and colleagues.[16] That study, albeit involving allogeneic tissue

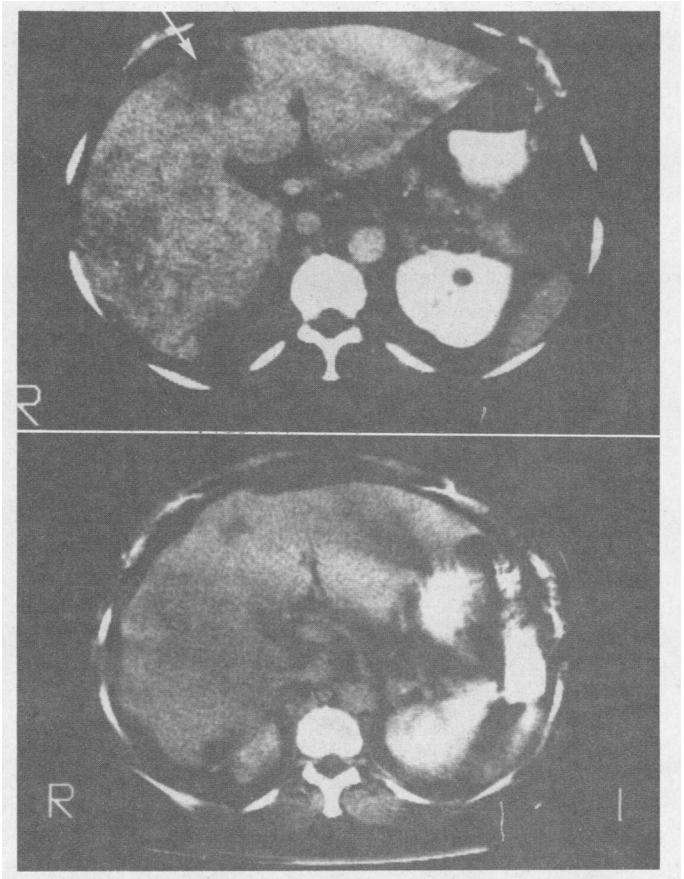

Figure 3.—Top, A computed tomogram taken of patient 32 shows an atrial myxoma **(arrow)** invading major vessels. **Bottom,** 70 days later, there is regression of the tumor nodule in the anterior lobe of the liver, with regeneration of liver tissue locally and in the posterior area of the right lobe.

culture cells, identified responses in 9 of 34 patients when intralymphatic therapy was the first method used but no responses in 13 patients who had received previous chemotherapy.[16] In contrast, five of our seven responders had previous chemotherapy.

Although we describe an evolving strategy for implementing active specific intralymphatic immunotherapy, the role of cyclophosphamide and of pretreatment of the tumor cells with cholesteryl hemisuccinate is still unclear. Skornick and associates reported responses in 7 of 21 patients treated with cholesteryl-modified autologous tumor cells.[17] This process did not amplify the response rate in our study. The incidence of clinical responses is not meaningfully different among the three series: 3 of 13 (23%) in series 1, 2 of 7 (29%) in series 2, and 2 of 12 (17%) in series 3.

The method and experience described suggest the existence of host responses that can have potentially useful effects against disseminated malignancy. Attempts to identify the existence of relevant serum antibodies, described by Ahn and co-workers[18] and by Fareed and colleagues,[19] have been unrewarding to date, both in our own laboratory and in an investigation by Karen G. Barnett, PhD, Hybridtech, Inc, San Diego (oral communication, September 1987). We have previously reported an initial impression that intralymphatic immunotherapy produces substantial augmentation of the CD4+ T-cell phenotype.[9,11] A further analysis indicates that this impression is confirmable,[20] and, notably, the more substantial increases of this subset also correlated significantly with the clinical response. Immunologic studies in progress may uncover or clarify further the underlying host-defense mechanisms and will be further evaluated in a separate report.

Our experience suggests that the method of intralymphatic immunotherapy is reasonably safe and technically feasible. It is hard to explain the regressions observed by mechanisms other than host immune responses engendered by the stimulus of the intralymphatic vaccine; understanding this process and modifying it to provide better and more durable responses remain a compelling challenge for further work.

Although one of us (C.L.W.) had previously initiated a similar program at a major academic center, we had some initial concerns about the feasibility of implementing this novel investigation at a community hospital. These concerns were not manifest in reality, and the community support of the program was gratifying. How best to further evaluate the method and to introduce it into clinical practice deserves attention. We are currently engaged in a clinical trial to evaluate a possible role for interleukin 2 in conjunction with this technique of active specific intralymphatic immunotherapy.

REFERENCES

1. Wiseman C, Rao VS: Tumor immunology and immunotherapy, chap 46, *In* McKenna RJ, Murphy GP (Eds): Fundamentals of Surgical Oncology. New York, Macmillan, 1986, pp 936-954

2. Juillard GJF, Boyer PJ, Yamashiro CH: A phase I study of active specific intralymphatic immunotherapy (ASILI). Cancer 1978; 41:2215-2225

3. Fisher B, Gollinger RC, Kelly M, et al: Variation of macrophage migration by a factor from regional lymph node cells of breast cancer patients. Cancer 1978; 42:2096-2100

4. Hoon DSB, Bowker RJ, Cochran AJ: Suppressor cell activity in melanoma-draining lymph nodes. Cancer Res 1987; 47:1529-1533

5. Juillard GJF, Boyer PJJ, Snow HD: Intralymphatic infusion of autochthonous tumor cells in canine lymphoma. Int J Radiat Oncol Biol Phys 1976; 1:497-503

6. Jeglum KA, Young KM, Barnsley K, et al: Intralymphatic autochthonous tumor cell vaccine in canine lymphoma. J Biol Response Mod 1986; 5:168-175

7. Adler A, Gillon G, Lurie H, et al: Active specific immunotherapy of renal cell carcinoma patients: A prospective randomized study of hormono-immuno- versus hormonotherapy. J Biol Response Mod 1987; 6:610-624

8. Adler A, Stein JA, Goldfarb AJ, et al: Active specific immunotherapy of stage III breast cancer: Result of an exploratory study. Cancer Immunol Immunother 1980; 10:45

9. Wiseman C, Rao VS, Bakke A, et al: Increased T-helper lymphocytes following active specific intralymphatic immunotherapy of cancer. J Biol Response Mod 1986; 5:490-497

10. Rao VS, Wiseman C, Mazumder A, et al: Effect of cholesteryl hemisuccinate (CHS) in cell-mediated immunity in melanoma patients treated with active specific intralymphatic immunotherapy. Proc Am Assoc Cancer Res 1988; 29:409

11. Rao VS, Wiseman C, Udis B, et al: Partial characterization of two subpopulations of T4 cells induced by active specific intralymphatic immunotherapy in melanoma patients. Proc Am Assoc Cancer Res 1986; 27:325

12. Berd D, Mastrangelo MJ: Effect of low dose cyclophosphamide on the immune system of cancer patients: Depletion of CD4+, 2H4+ suppressor-inducer cells. Cancer Res 1988; 48:1671-1675

13. Skornick Y, Danciger E, Rozen R, et al: Positive skin tests with autologous tumor cells of increased membrane viscosity: First report. Cancer Immunol Immunother 1981; 11:93-96

14. § 610.12. Federal Register 1973; 38:32056

15. McCune CS, Schapira DV, Henshaw EC: Specific immunotherapy of advanced renal carcinoma: Evidence for the polyclonality of metastases. Cancer 1981; 47:1984-1987

16. Weisenburger TH, Jones PC, Ahn SS, et al: Active specific intralymphatic immunotherapy in metastatic malignant melanoma: Evidence of clinical response. J Biol Response Mod 1982; 1:57-66

17. Skornick YG, Rong GH, Sindelar WF, et al: Active immunotherapy of human solid tumor with autologous cells treated with cholesteryl hemisuccinate—A phase I study. Cancer 1986; 58:650-654

18. Ahn SS, Irie RF, Weisenburger TH, et al: Humoral immune response to intralymphatic immunotherapy for disseminated melanoma: Correlation with clinical response. Surgery 1982; 92:362-367

19. Fareed GC, Mendiaz E, Sen A, et al: Novel antigenic markers of human tumor regression. J Biol Response Mod 1988; 7:11-23

20. Wiseman C, Rao S, Kennedy P, et al: Biological correlates of objective response in autologous active specific intralymphatic immunotherapy in human malignancy (AASILI). Proc Soc Biol Ther 1988; III-7:19